

represent the bound [ $^{125}$ I] TNFa in the presence of 180 fold excess of cold unlabeled TNFa.

Figure 43 describes the cytotoxicity assay of an HPLC RPC-8 fraction of the human monocytes which were treated with PMA and PHA for 24 hours.

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cont'd  
Figures 44A to 44C describe the RPC-8 chromatographic pattern of 40kDa TNF inhibitor  $\Delta 51$  (A), SDS-polyacrylamide gel analysis of the fractions (B), and the cytotoxicity assay on L929 cells (C).

Figures 45A to 45C describe the RPC-8 chromatographic pattern of 40kDa TNF inhibitor  $\Delta 53$  (A), SDS-polyacrylamide gel analysis of fractions (B), and the cytotoxicity assay on L929 cells (C).

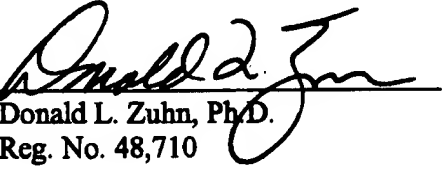
#### REMARKS

The Applicants contend that no new matter has been added as a result of the above-described amendments. The amendments merely correct any improper reference to the figures made in the specification. The Applicants request that the Examiner direct any inquiries regarding this communication to the undersigned representative at 312-913-0001.

Respectfully submitted,  
McDonnell Boehnen Hulbert & Berghoff

Dated: January 2, 2003

By:

  
Donald L. Zuhn, Ph.D.  
Reg. No. 48,710



## **AMENDMENTS TO THE SPECIFICATION**

### **Marked Up Version of Replacement Paragraphs of Specification**

#### **under 37 C.F.R. 1.121(b)(1)(iii)**

Please amend the section at page 5, line 15 to page 9, line 37 to read as follows:

Figure 1 describes a cytotoxicity assay for TNF in the absence (-.-) and in the presence (-x-x-) of TNF inhibitor (30kDa). Various concentrations of TNF were incubated with and without TNF inhibitor, and the cytotoxicity assay was performed as described in Example 1.

Figure 2 describes a native gel shift assay in which "a" depicts TNF alone, and "b" depicts TNF + TNF inhibitor (30kDa).

Figure 3 describes Con A-Peroxidase staining of TNF inhibitor (30kDa). About 200 ng of each protein were run on 14% SDS-PAGE, and transferred to nitrocellulose filter. Glycoproteins were identified using Con A-peroxidase staining. In this figure, "a" depicts a molecular weight marker, "b" depicts Ovalbumin, "c" depicts bovine serum albumin, and "d" depicts TNF inhibitor.

Figure 4 describes chemical deglycosylation of TNF inhibitor (30kDa). About 200 ng of TNF inhibitor were chemically deglycosylated (lane C) as described in Example 1.

Figure 5 describes N-glycanase treatment of TNF inhibitor (30kDa). Purified TNF inhibitor was iodinated by Bolton-Hunter reagent, and denatured-iodinated TNF inhibitor was treated with N-glycanase for 6 hours at 37°C. In this figure, "a" depicts native TNF inhibitor, and "b" depicts deglycosylated TNF inhibitor.

Figure 6A describes an OD<sub>280</sub> profile of the DEAE Sepharose CL-6B chromatography of 20 l urine.

Figure 6B describes an autoradiograph of the corresponding native gel shift assay indicating a peak of TNF inhibitor at fraction 57-63, which is about 80mM NaCl.

Figure 7 describes an OD<sub>280</sub> profile of the 0.05-M Na Phosphate pH 2.5 elution from the TNF affinity column.

Figures 8A and 8C describe chromatographic profiles (OD<sub>215</sub> and OD<sub>280</sub>) of the RP8 purification of the TNF inhibitor (30kDa) with the L929 bioassay of fractions from the RP8

column showing a peak of TNF inhibitor at fractions 28-31 which is about 18% acetonitrile and at fractions 35 and 36 which is about 21% acetonitrile.

Figure 8B describes a silver stained 15% SDS-PAGE of the RP8 pool showing a single band at 30kDa.

Figure 9A describes a peptide purification of Lys-C digestion of TNF inhibitor (30kDa).

Figures 9B-1 to 9B-3 describes a peptide purification of alkylated (\*) Lys-C digests of TNF inhibitor (30kDa).

Figures 10A and 10B describes a peptide purification of two alkylated (\*) Asp-N digests of TNF inhibitor (30kDa).

Figures 11A and 11B describe peptide purifications of an endopeptidase V8 digest of reduced carboxymethylated TNF inhibitor (30kDa).

Figure 12 describes amino acid sequences present in TNF inhibitor (30kDa). Blanks in the sequence indicate the residue has not been unambiguously identified by protein sequencing. C\* indicates the identification of carboxymethylcysteine by the presence of  $^3\text{H}$  in the residue.

Figures 13A to 13C describes the DNA sequence of a genomic clone encoding at least a portion of a TNF inhibitor (30kDa).

Figure 14 describes at least 70% of the mature amino acid sequence of a preferred TNF inhibitor (30kDa).

Figure 15 describes detection of TNF inhibitor in U937 supernatant by the gel shift assay.

Figure 16 describes detection of TNF inhibitor in hplc fractions from U937 supernatant.

Figure 17 describes the Northern blot according to Example 4.

Figure 18 describes the deglycosylated TNF inhibitor (30kDa) binding to TNF.

Glycosylated and deglycosylated TNF inhibitor were incubated with TNF affigel, and flow through materials and eluates of the gel were analysed on SDS-PAGE. In this figure, (11) indicates flow through of TNF-INH, reduced and oxidized, (21) indicates flow through of deglycosylated TNF-INH, reduced and oxidized, (51) indicates flow through of native TNF-INH, (12) indicates eluate of TNF-INH, reduced and oxidized, (22) indicates eluate of deglycosylated TNF-INH, reduced and oxidized, and (52) indicates eluate of native TNF-INH.

Figure 19 describes the complete amino acid sequence of the 30kDa TNF inhibitor.

Figures 20A and 20B describes the cDNA sequence encoding the amino acid sequence

shown in Figure 19.

Figures 21A to 21F describes the entire cDNA sequence for the precursor of the 30kDa TNF inhibitor.

Figure 22 describes the DNA sequence near the start of the TNF inhibitor (30kDa) gene in plasmid pTNFIX-1.

Figure 23 describes the plasmid pCMVXV beta TNFBP stop A.

Figure 24 describes the plasmid pSVXVTNFBP stop A.

Figure 25 describes a chromatographic profile  $OD_{215}$  of the RP8 column of the 30kDa TNF inhibitor from E. Coli. The L929 bioassay results are also shown (-x-x).

Figure 26 describes a silver stained 14% SDS-PAGE of the RP8 Fractions in Figure 25.

Figure 27 describes a chromatographic profile ( $OD_{215}$ ) of the RP8 purification of the TNF inhibitors from U937 cells. The L929 bioassay results are also shown with a bar graph. Two distinctive TNF inhibitor peaks are seen.

Figure 28 describes a silver stained 14% SDS-PAGE of the RP8 fractions. Fraction number 30 contains the 30kDa TNF inhibitor and fraction number 35 contains the 40kDa TNF inhibitor.

Figure 29 describes a chromatographic profile ( $OD_{215}$ ) of the purification of urinary 40kDa TNF inhibitor. The second TNF inhibitory peak from several RP8 chromatographies were combined and reanalyzed on an RP8 column. TNF-inhibitory activity is shown with a bar graph. The difference between the  $OD_{215}$  peak and the activity peak reflects the dead volume between the detector and the fraction collector.

Figure 30 describes a silver stained 14% SDS-PAGE of the RP8 fractions of urine. Fraction number 32 contains the 40kDa TNF inhibitor.

Figure 31 describes the amino terminal sequences of U937 derived inhibitors (30kDa and 40kDa), and urine-derived 40kDa TNF inhibitor.

Figure 32 describes a peptide purification of endopeptidase V8 digested 40kDa TNF inhibitor.

Figure 33 describes a peptide purification endopeptidase Arg-C digested 40kDa TNF inhibitor.

Figure 34 describes a peptide purification of trypsin digested Arg-C16 peptide.

Figure 35 describes a peptide purification of chymotrypsin digested Arg-C10 peptide.

Figure 36 describes a primary structure of the 40kDa TNF inhibitor.

Figure 37 describes a portion of the 40kDa TNF inhibitor cDNA sequence along with the predicted amino acid translation product.

Figure 38 describes the complete amino sequence of the 40kDa TNF inhibitor.

Figures 39A to 39H describes the entire cDNA sequence for the precursor of the 40kDa TNF inhibitor, along with its deduced translation product.

Figure 40 describes a cytotoxicity assay for TNF beta (lymphotoxin) in the ~~presense~~ presence (o-o) of 40kDa TNF inhibitor, in the ~~presense~~ presence (o-o) of 30kDa TNF inhibitor and without any inhibitor (x-x).

Figure 41 describes the expression of the 30kDa TNF inhibitor cDNA sequence shown in Figure 21 in COS7 cells. COS cells were transfected with plasmids using the lipofection procedure of Feigner *et al.* (*Proc. Natl. Acad. Sci. (USA)* 84, 7413-1987) Feigner *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:7413-17.  $3.4 \times 10^5$  cells were incubated with the indicated amounts of [ $^{125}$ I] TNFa at a specific activity of  $5.6 \times 10^4$  cpm/ng and the amount bound to the cells determined. Open symbols are the total cpm associated with cells after a 4 hour incubation at 4°C. Closed symbols represent bound [ $^{125}$ I] TNFa in the ~~presense~~ presence of 180 fold excess of cold unlabeled TNFa.

Figure 42 describes the expression of the 40kDa TNF inhibitor cDNA sequence shown in Figure 39 in COS7. Assay conditions were as described in Figure 41. The darkened symbols represent the bound [ $^{125}$ I] TNFa in the ~~presense~~ presence of 180 fold excess of cold unlabeled TNFa.

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Figures 44A to 44C describes the RPC-8 chromatographic pattern of 40kDa TNF inhibitor  $\Delta 51$  (A), SDS-polyacrylamide gel analysis of the fractions (B), and the cytotoxicity assay on L929 cells (C).

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